

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 37/36, 47/12	A1	(11) International Publication Number: WO 93/19776 (43) International Publication Date: 14 October 1993 (14.10.93)
(21) International Application Number: PCT/SE93/00281 (22) International Filing Date: 1 April 1993 (01.04.93) (30) Priority data: 9201073-5 3 April 1992 (03.04.92) SE (71) Applicant (for all designated States except US): KABI PHARMACIA AB [SE/SE]; S-751 82 Uppsala (SE). (72) Inventors; and (75) Inventors/Applicants (for US only) : CASTENSSON, Stefan [SE/SE]; Vårbroddvägen 5, S-741 42 Knivsta (SE). FLORIN-ROBERTSSON, Ebba [SE/SE]; Norrtullsgatan 12 E, S-113 27 Stockholm (SE). HÖKBY, Elvy [SE/SE]; Sicklingsvägen 6, S-122 46 Enskede (SE). THOME, Sirkka [SE/SE]; Myrstuguvägen 359, lg 360, S-143 00 Värby (SE).		(74) Agents: TANNERFELDT, Agneta et al.; Kabi Pharmacia AB, S-112 87 Stockholm (SE). (81) Designated States: AU, CA, FI, JP, NO, NZ, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: PROTEIN FORMULATION COMPRISING GROWTH HORMONE (57) Abstract The invention relates to injectable formulations of growth hormone or any functional analogue thereof, comprising citrate as buffer substance. The formulation could be an aqueous solution of growth hormone or any functional analogue thereof and citrate as buffer substance in a concentration of 2-50 mM. The formulation of the growth hormone or any functional analogue thereof could comprise growth factors, amino acids such as e.g. glycine and alanine and/or mannitol or other sugar alcohols and/or glycerol and/or other carbohydrates and optionally a preservative such as benzyl alcohol. The invention also relates to a process for preparation of the formulation by mixing growth hormone or any functional analogue thereof with citrate as buffer substance or by adding the constituents of the final formulation on the last gel purification step.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SU	Sudan
CG	Congo	KK	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TC	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

PROTEIN FORMULATION COMPRISING GROWTH HORMONE

5 The present invention relates to formulation of growth hormone or any functional analogue thereof, especially human growth hormone (hGH) in solutions, which comprise citrate in an concentration of about 2 to 50 mM as buffer substance and especially sodium citrate in an concentration of about 2 to 20 mM. This buffer is used for
10 improving stability.

Growth hormone can be both human and animal such as human growth hormone (hGH), bovine growth hormone (bGH) and porcine growth hormone (pGH).

15 hGH is a protein consisting of a single chain of 191 amino acids. The molecule is cross-linked by two disulphide bridges and the monomeric form has a molecular weight of 22 kDa. However, pituitary human growth hormone is not homogeneous. For example, a
20 smaller 20 kDa hGH variant produced from the same gene is also known. The "basic hGH" variant (hGH-V) expressed by the placenta during pregnancy is another analogue which is a product of a separate gene. Like the 22 kDa hGH it consists of 191 amino acids but in various positions throughout the molecule 13 of them are different.
25 See e.g. Bewley TA et al; Adv Enzymol; 42; 73-166; 1975 and Frankenne F et al; J Clin. Endocrin and Metabol; 66; 1171-80; 1988.

Recombinant hGH (22 kDa) has been commercially available for several years. It is preferred over the pituitary derived products
30 because the product prepared from human tissue might contain infectious agents such as that for the Creutzfeld-Jacob's disease. Two types of therapeutically useful recombinant hGH preparations are present on the market: the authentic one, e.g. Genotropin®, Kabi Pharmacia AB, and an analogue with an additional methionine residue
35 at the N-terminal end, e.g. Somatonorm®.

hGH is used to stimulate linear growth in patients with hypopituitary dwarfism or Turner's syndrome but other indications have also been suggested.

5 The stability of proteins in aqueous formulations is generally a problem in pharmaceutical industry.
It has often been solved by drying of the protein in different drying processes, such as freeze-drying. The protein has thereafter been distributed and stored in dried form. The patient necessarily has to
10 reconstitute the dried protein in a solvent before use, which of course is a disadvantage and is an inconvenience for the patient.

By new devices for administrations, e.g. Kabipen® which is described in US 4 968 299, Kabi Pharmacia AB, the patient has got a device
15 which is rather simple to handle. The device comprises a two-chamber ampoule Genomix® containing hGH as a lyophilised powder in one of the compartments and a reconstitution diluent in the other. The patient reconstitutes the product before use. The lyophilised product can be stored for up to 24 months. The reconstituted product
20 is then stable for 3 weeks when stored at 2-8°C.

The freeze-drying process is a costly and time consuming process step, and it would be of great advantage if this step could be avoided, when preparing a commercial product of a protein.

25 For a patient, who needs daily injections of a growth hormone e.g. hGH, and especially when the patient is a child, it is of importance that the product is easy to handle, to dose and inject. The reconstitution of freeze-dried hGH demands prudence and carefulness
30 and should preferably be avoided, but is the only method available today.

It would facilitate the use of growth hormone and especially hGH, if the protein could be produced and distributed as a solution to the
35 patient, who could inject the medicament directly without reconstitution.

Different solutions to this problem have been disclosed, but until now no product has appeared on the market.

5 In WO 89/09614, Genentech, a stabilised formulation of hGH comprising glycine, mannitol and a buffer is disclosed and in a preferred embodiment a non-ionic surfactant such as polysorbate 80 is added. Sodium-phosphate is suggested as buffer substance. The formulation has an increased stability in a lyophilised formulation and upon reconstitution.

10

Another possibility of administering growth hormone in a solution is to add a block copolymer containing polyoxyethylene-polyoxypropylene according to EP 211 601, International Minerals and Chemical Corporation. This solution provides for a prolonged release upon administration to the animal.

15

There is a demand on the market for stabilised, injectable growth hormone solutions, e.g. hGH in a solution. It would also be advantageous if the final pharmaceutical solution only contained a minimum of additives, such as tensides.

20

We have now found a new formulation which solves the above mentioned problems.

25

GH STABILITY

The stability of hGH depends on the chemical and physical properties of the protein.

30 Different degradation pathways are known such as deamidation, oxidation and aggregation.

Deamidation and oxidation are common chemical reactions comprising changes of the primary structure of the protein.

35 Deamidation occurs especially in aqueous solutions but low temperature and low pH of the solutions suppress the deamidation reaction.

Different forms of aggregation result from the physical instability of the protein. Aggregates can be soluble or insoluble and binding of both the forms can be covalent or non covalent.

5 The aggregates can give opalescent solutions but there can also be non-visible aggregation which only can be shown chemically.

The prevention of covalent aggregation in protein formulations is of importance since such processes are irreversible and could result in the production of inactive species which in addition also may be
10 immunogenic.

Changes in the primary structure may also give rise to conformational changes which can be the cause of self association of the protein, aggregation.

The non covalent aggregation occurring under certain conditions can
15 lead to precipitation and loss of activity.

A large number of reactions can occur under different pH conditions and it is almost impossible to formulate a protein at a particular pH that eliminates all the modification reactions while maintaining high
20 solubility and proper conformation of the protein.

Until now a slightly alkaline pH has generally been used by manufacturers to avoid visible particles and to obtain a clear product. In most commercial products the pH is over 7, in spite of the
25 higher risk for deamidation.

When Kabi Pharmacia's product Genotropin® is reconstituted, a pH of 6.7 is obtained at a hGH concentration of 16 IU/ml. This pH is a compromise between a pH giving a totally clear solution (pH 8) and
30 pH 6 giving a lower deamidation rate but somewhat more opalescence.

Because of this complexity it is not possible to formulate a protein preparation and eliminate all the degradation pathways. The freeze-dried protein product is much more stable than the corresponding
35 aqueous solution. However, although a freeze-dried product is

sufficiently stable after processing, the protein will still degrade slowly during storage.

5 THE INVENTION

Totally unexpected we have now found that solutions containing growth hormone in which citrate has been chosen as a buffer substance are more stable than those in which phosphate is the buffer.

10

The invention relates to injectable formulations of growth hormone or any functional analogue thereof, comprising citrate as buffer substance.

15

The formulation could be an aqueous solution of growth hormone or any functional analogue thereof and citrate as buffer substance in a concentration of 2-50 mM and preferably contains sodium citrate as buffer substance in a concentration of 2-40 mM at a pH of about 5.0 to 7.5.

20

Preferably the formulation is an aqueous solution of hGH or any functional analogue thereof and citrate as buffer substance in a concentration of 2 to 20 mM, e.g. 5 mM or 10 mM and preferably contains sodium citrate as buffer substance at a pH of about 6.0 to 7.0.

25

The formulation of the growth hormone or any functional analogue thereof could comprise amino acids such as e.g. glycine and alanine and/or mannitol or other sugar alcohols and/or glycerol and/or other carbohydrates and optionally a preservative such as benzyl alcohol.

30

The solution should be isotonic. The growth hormone is preferably recombinant hGH.

35

The formulation according to the invention is stable for at least 12 months. By stable is here meant an amount of more than 85 % monomer (IEF) and fragments according to SDS-PAGE of less than 2 %.

The claimed formulation can also comprise growth hormone and growth factors in a mixture.

By growth factor is meant insulin-like growth factors (IGF-1 or IGF-2) and epidermal growth factor (EGF), either from natural sources or
5 produced by recombinant techniques.

The invention also relates to a process for preparation of the formulation by mixing growth hormone or any functional analogue thereof with citrate as buffer substance or by adding the
10 constituents of the final formulation on the last gel purification step.

It also relates to a method for treatment of a patient in need of growth hormone any functional analogue thereof by administering the claimed formulation.
15

By growth hormone (GH) is meant both naturally occurring human and animal GH and recombinant GH (rGH), such as rhGH, rbGH and rpGH. By functional analogues are meant compounds having the same therapeutic effect as the growth hormone in animals and humans.
20

The growth hormone to be included in the buffer could be the initially isolated product or a product which has been freeze-dried and thereafter reconstituted.

25 The concentration of the growth hormone is only dependent of its solubility in the used buffer and the desired therapeutically amount for the given dose. Preferably the concentration of hGH is 1 - 80 IU/ml and more preferably 2 - 40 IU/ml.

30 Aggregation in the form of precipitation observed by shaking the solution, is a result of denaturation at the air - liquid interface. This could be avoided by filling without headspace, thus diminishing the condition for aggregation.

35

EXAMPLE 1.

Material for formulation studies was obtained from the ordinary Genotropin® process. The human growth hormone is synthesized in the bacterium *Escherichia coli* (E coli) K12, using the expression plasmid pAPSTIIhGH-3 as template. The hormone is secreted out from the cell into the periplasmic space during synthesis. Human growth hormone is subsequently isolated from the E coli periplasmic space after disruption of the outer bacterial membrane.

The extract containing recombinant hGH was fractionated on DEAE-Sepharose FF. Two ammonium sulphate precipitation steps followed and thereafter two fractionation steps on DEAE-Sepharose FF.

Formulation was performed by gel filtration which serves the purposes of removing salts used in the previous purification steps and adding the constituents of the final formulation. The last column Sephadex G-25 (Pharmacia, diameter 1.3 cm, bed height 45 cm) was equilibrated with the formulation buffer. Equilibration and chromatography was performed at +7°C.

The desired protein concentration was achieved by diluting with the formulation buffer.

The stability of seven solutions was investigated. See table 1.

Table 1

Example	A	B	C	D
hGH IU/ml	20	20	10	10
Na-citrate, mM	5	-	-	-
Na-phosphate, mM	-	5	10	10
glycine, mM	12	12	12	12
mannitol, mM	250	250	250	250
pH	6,2	6,3	6,2	7,4
Volume	1	1	1	1
Starting values:				
pH	6,2	6,3	6,2	7,4
IEF (% monomer)	98	98	99	100
SDS-PAGE				
aggregates, %	0	0	-	-
monomer, %	99,8	99,6	-	-
fragments, %	0,2	0,4	-	-
visual inspect.	clear	clear	clear	clear

Table 1 Cont.

Example		A	B		
The results after 6 months' storage at 5°C:					
	pH	6,3	6,4	6,4	7,5
5	IEF (% monomer)	88	84	88	73
SDS-PAGE					
	aggregates, %	0	0	0	0,2
	monomer, %	99,7	95,1	97,5	98,4
	fragments, %	0,3	4,9	2,5	1,3
10	visual inspect.	clear	-		

The results after 15 months' storage at 5°C:					
15	pH	6,3	6,4		
	IEF (% monomer)	86	75		
SDS-PAGE					
	aggregates, %	0	0,1		
	monomer, %	98,9	92,7		
20	fragments, %	1,0	7,3		
	visual inspect.	clear	clear		

The results after 24 months' storage at 5°C:					
25	pH	6,4	6,4		
	IEF (% monomer)	88	71		
SDS-PAGE					
	aggregates, %	0	0		
	monomer, %	98,3	89,1		
30	fragments, %	1,7	10,9		
	visual inspect.	clear	clear		

Table 2

35	Example	E	F	G	H
	hGH IU/ml	4	4	10	10
	Na-citrate, mM	10	-	-	5
40	Na-phosphate, mM	-	10	10	-
	glycine, mM	12	12	12	12
	mannitol, mM	250	250	250	250
	pH	6,2	6,1	6,3	6,1
	Volume	3	3	2	0.35

Table 2 Cont.

Example	E	F	G	H
5 Starting values:				
pH	6,2	6,1	6,3	6.1
IEF (% monomer)	99	99	97	100
visual inspect.	clear	clear	clear	clear
10				
The results after 6 months' storage at 5°C:				
pH	6,3	6,2	6,3	6.6
15 IEF (% monomer)	89	86	85	91
SDS-PAGE				
aggregates, %	0	0	0.1	0
monomer, %	99.8	97.1	95.2	98.1
fragments, %	0.2	2.9	4.7	1.9
20 visual inspect.	clear	clear	clear	clear
The results after 12 months' storage at 5°C:				
pH			6.3	6.5
25 IEF (% monomer)			71	90
SDS-PAGE				
aggregate, %			0,4	0
monomer, %			93	98.1
fragments, %			6.6	1.8
30 visual inspect.			-	clear

METHODS

35 Isoelectric focusing (IEF) with densitometric evaluation

IEF is a method according to which the extent of deamidation can be evaluated.

The separation of hGH components is carried out in a pH gradient, which is established between two electrodes and stabilised by carrier ampholytes. The proteins migrate until they align themselves at their isoelectric point in the gradient, at which a protein possesses no net overall charge and will therefore concentrate as migration ceases. Thus the separation is obtained according to

charge. The relative distribution of charged hGH forms are quantified by densitometric scanning of Coomassie Blue stained polypeptides. The higher percentage of the monomer, the less deamidation.

5 Polypeptides size distribution (SDS-PAGE)

Proteins in preparations of somatropin, hGH, were denatured by sodium dodecyl sulphate (SDS) to yield negatively charged molecular complexes of SDS-protein. Separation was then obtained according to molecular size by electrophoresis in polyacrylamide gels (PAGE) in the presence of SDS. The relative polypeptide size distribution of hGH was quantified by densitometric scanning of the silver stained polypeptide bands.

15 Visual inspection

The appearance of the solutions were eye-inspected according to Ph. Eur. 2nd Ed.

20 pH

pH was measured with glass and calomel electrodes.

Examples A, E and H are according to the invention.

From tables 1 and 2 it is clearly seen that the percentage of fragment is much higher in the solution buffered with Na-phosphate than with Na-citrate.

25 B (Na-phosphate) contains after 15 months' storage at 50°C 7.3 % fragments and 92.7 % of the monomer and A (Na-citrate) has 1.0 % and 98.9 % respectively.

30 For F (Na-phosphate) the relative amount of fragment is 2.9 % and the percentage of monomer is 97.1 % after 6 months' storage at 50°C. E (Na-citrate) has 0.2 % and 99.8 %, respectively .

B, C, D, F and G contain Na-phosphate and all have high percentage of fragments after storage.

35 D has a higher pH, but the amount of fragments is higher than for the solution E using 10 mM Na-citrate buffer.

The grade of deamidation is unacceptably high in D.

EXAMPLE 2

This example was performed in order to compare compositions according to the invention with and without benzyl alcohol.
See table 3

The formulations were prepared in the same way as explained in example 1, but the formulation buffer solution contained benzyl alcohol.

Table 3

Example	I	K	L	A
hGH IU/ml	20	20	20	20
Na-citrate, mM	10	10	5	5
glycine, mM	12	-	12	12
mannitol, mM	150	150	130	250
benzyl alcohol %	1	1	1	-
pH	6.3	6.3	6.3	6.2
Volume	1	1	3.5	1

The result after 3 weeks' storage at 30°C:

Example	I	K	L	A
pH	6.3	6.3	6.2	6.2
IEF (% monomer)	67	68	64	72
clarity	clear	clear	clear	clear

The result after 1 month's storage at 5°C:

pH	6.3	6.3	6.2	6.2
IEF (% monomer)	99	99	-	97
clarity	clear	clear	clear	clear

The results after 3 months' storage at 5°C

pH	6.3	6.4	6.2	6.3
IEF (% monomer)	94	94	96	94
clarity	clear	clear	clear	clear
SDS-PAGE)				
aggregates, %			0	0,1

EXAMPLE 2

This example was performed in order to compare compositions according to the invention with and without benzyl alcohol.

See table 3

5

The formulations were prepared in the same way as explained in example 1, but the formulation buffer solution contained benzyl alcohol.

10 Table 3

Example	I	K	L	A
hGH IU/ml	20	20	20	20
Na-citrate, mM	10	10	5	5
glycine, mM	12	-	12	12
mannitol, mM	150	150	130	250
benzyl alcohol %	1	1	1	-
pH	6.3	6.3	6.3	6.2
Volume	1	1	3.5	1

20

The result after 3 weeks' storage at 30°C:

Example	I	K	L	A
pH	6.3	6.3	6.2	6.2
IEF (% monomer)	67	68	64	72
clarity	clear	clear	clear	clear

25

The result after 1 month's storage at 5°C:

pH	6.3	6.3	6.2	6.2
IEF (% monomer)	99	99	-	97
clarity	clear	clear	clear	clear

30

35 The results after 3 months' storage at 5°C

pH	6.3	6.4	6.2	6.3
IEF (% monomer)	94	94	96	94
clarity	clear	clear	clear	clear
SDS-PAGE)				
aggregates, %			0	0,1
monomer, %			99.8	99.4
fragments, %			0.2	0.5

40

5 Example 2 is performed to show how the addition of benzyl alcohol influences stability when Na-citrate is used as buffer.

Benzyl alcohol is a preservative which is conditional to use in injectable multidose preparations.

10 According to the pharmacopoeias it is a demand that a suitable preservative is added to injectable multidose preparations in order to guarantee the microbial safety of the product.

As benzyl alcohol has an influence on the isotonicity the amount of mannitol is adjusted accordingly when benzyl alcohol is added.

15 From Table 3 it is clearly seen that the addition of benzyl alcohol has no influence on stability when Na-citrate is used as buffer.

EXAMPLE 3

20 This example was performed in order to compare compositions according to the invention with and without glycine and mannitol. See table 4

25 The formulations were prepared in the same way as explained in example 1.

Table 4

30	Example	M	N
	hGH IU/ml	20	20
	Na-citrate, mM	5	5
	glycine, mM	-	12
	mannitol, mM	-	150
35	pH	6.3	6.3
	Volume	1	1

Table 4- Cont

Example		M	N
5			
The result after 1 weeks' storage at 30°C:			
	pH	6.3	6.3
10	IEF (% monomer)	92	91
	SDS-PAGE		
	aggregates, %	0	0
	monomer, %	99.5	99.6
	fragments, %	0.5	0.4
15	visual inspect.	clear	clear

From Table 4 it is seen that the addition of additives such as glycine and mannitol has no influence on stability when Na-citrate is used as buffer.

CLAIMS

- 5
1. Injectable formulation of human growth hormone or any functional analogue thereof, comprising citrate in an amount of 2-50 mM as buffer substance.
 - 10 2. Formulation according to claim 1 in which the formulation is an aqueous solution of a growth hormone or any functional analogue thereof.
 - 15 3. Formulation according to claim 2 in which the formulation is an aqueous solution of hGH.
 - 20 4. Formulation according to claim 3 in which the formulation is an aqueous solution of hGH and citrate as buffer substance in a concentration of 2 to 20 mM.
 - 25 5. Aqueous solution of hGH according to any of claims 2-4 comprising sodium citrate as buffer substance and pH of about 6 -7.
 6. Aqueous solution of hGH according to claim 5, comprising sodium citrate in an amount of 5 mM.
 - 30 7. Aqueous solution of hGH according to claim 5, comprising sodium citrate in an amount of 10 mM.
 - 35 8. Formulation of growth hormone or any functional analogue thereof according to claim 1 or 2, comprising one or several growth factors.
 9. Formulation of growth hormone or any functional analogue thereof according to claim 1, 2 or 8, comprising glycine and/or mannitol, and/or glycerol.

10. Formulation of growth hormone or any functional analogue thereof according to any of claim 1 to 9 comprising a preservative.
- 5 11. Formulation of growth hormone according to claim 10 which is an aqueous solution of hGH and the preservative is benzyl alcohol.
12. Formulation of growth hormone according to claim 3 in which hGH is recombinant hGH.
- 10 13. Process for preparation of a formulation according to any of claims 1-12, characterised by mixing growth hormone or any functional analogue thereof with citrate as buffer substance or by adding the constituents of the final formulation on the last gel purification step.
- 15 14. Method for treatment of a patient in need of growth hormone or any functional analogue thereof by administering a formulation according to any of claims 1-12.

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 93/00281

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 37/36, A61K 47/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, EMBASE, MEDLINE, WPI, CHEMICAL ABSTRACTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, A1, 0303746 (INTERNATIONAL MINERALS AND CHEMICAL CORPORATION), 22 February 1989 (22.02.89) --	1-13
X	Chemical Abstracts, Volume 113, No 2, 9 July 1990 (09.07.90), (Columbus, Ohio, USA), page 374, THE ABSTRACT No 12165x, JP, A, 1308235, (KAGAYA, S et al) 12 December 1989 (12.12.89) --	1-13
X,P	WO, A2, 9217200 (GENENTECH, INC.), 15 October 1992 (15.10.92) --	1-13
X	WO, A1, 9118621 (GENENTECH, INC.), 12 December 1991 (12.12.91) --	8,9

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

9 July 1993

12 -07- 1993

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Elisabeth Carlborg
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 93/00281

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A1, 9115509 (CALIFORNIA BIOTECHNOLOGY INC.), .17 October 1991 (17.10.91) -----	1-13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 93/00281

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 14
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

28/05/93

International application No.
PCT/SE 93/00281

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0303746	22/02/89	SE-T3- 0303746 HU-A- 44706	28/04/88
JP-A- 1308235	12/12/89	NONE	
WO-A2- 9217200	15/10/92	AU-A- 1757092	02/11/92
WO-A1- 9118621	12/12/91	EP-A- 0536226	14/04/93
WO-A1- 9115509	17/10/91	AU-A- 7692591 EP-A- 0527781	30/10/91 24/02/93